

Cher, J. Z.B. et al. (2018) Alarmins in frozen shoulder: a molecular association between inflammation and pain. *American Journal of Sports Medicine*, 46(3), pp. 671-678. (doi:[10.1177/0363546517741127](https://doi.org/10.1177/0363546517741127))

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Deposited on: 03 January 2018

Alarmins in Frozen Shoulder

A Molecular Association Between Inflammation and Pain

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Background: The pathophysiological mechanisms behind proliferation of fibroblasts and deposition of dense collagen matrix in idiopathic frozen shoulder remain unclear. Alarmins (also known as danger signals) are endogenous molecules that are released into the extracellular milieu after infection or tissue injury and that signal cell and tissue damage.

Purpose: To investigate whether the presence of alarmins is higher in patients with idiopathic frozen shoulder than in control subjects.

Study Design: Controlled laboratory study.

Methods: Shoulder capsule samples were collected from 10 patients with idiopathic frozen shoulder and 10 patients with unstable shoulders (control). The samples were stained with hematoxylin and eosin (H&E) and analyzed by immunohistochemistry using antibodies against alarmin molecules including high-mobility group protein B1 (HMGB1), interleukin 33, S100A8, S100A9, and the peripheral nerve marker PGP9.5. Immunoreactivities were rated in a blinded fashion from “none” to “strong.” Immunohistochemical distribution within the capsule was noted. Before surgery, patient-ranked pain frequency, severity and stiffness, and the range of passive shoulder motion were recorded and statistically analyzed.

Results: Compared with control patients, patients with frozen shoulder had greater frequency and severity of self-reported pain ($P = .02$) and more restricted range of motion in all planes ($P < .05$). H&E-stained capsular tissue from frozen shoulder showed fibroblastic hypercellularity and increased subsynovial vascularity. Immunoreactivity of alarmins was significantly stronger in frozen shoulder capsules compared with control capsules ($P < .05$). Furthermore, the expression of the alarmin molecule HMGB1 significantly correlated ($r > 0.9$, $P < .05$) with frequency of severity of pain and extreme pain in patients with frozen shoulder compared with controls.

Conclusion: This study demonstrates a potential role for key molecular danger signals in frozen shoulder and suggests an association between the expression of danger molecules and the pain experienced by patients.

Keywords: frozen shoulder; alarmins; inflammation; pain; HMGB1; adhesive capsulitis; IL-33; S100

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One or more of the authors has declared the following potential conflict of interest or source of funding: Funding was received from Wellcome Trust and Arthritis Research UK.

Adhesive capsulitis or frozen shoulder is defined as a clinical condition restricting the passive and active movement of the shoulder joint in all directions, including flexion, abduction, and rotation.⁵ Frozen shoulder is the third most common cause of musculoskeletal disability in the United States and is more common in the fifth and sixth decades of life, affecting women more than men.²² Cases of frozen shoulder are typically categorized into 3 groups: idiopathic, primary, and secondary. Primary adhesive capsulitis is normally idiopathic but has associations with hyperthyroidism, hypothyroidism, diabetes, and Dupuytren disease, while secondary adhesive capsulitis is usually the sequela of previous trauma, including surgery or immobilization.¹⁸

Our current understanding of the underlying cause and pathogenesis of frozen shoulder remains limited. Histologic

studies have demonstrated fibroblastic and myofibroblastic proliferation in dense type I and III collagen matrix in the shoulder capsule, while capsular fibrosis and contracture have been suggested to stiffen the shoulder capsule and thus restrict range of motion.⁵ Histologically, frozen shoulder tissue is characterized by myofibroblasts and chronic inflammatory cells, including mast cell, T cells, B cells, and macrophages.^{4,16} Furthermore, cytokines such as interleukin 1 β (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF- α), which are known to drive inflammatory and matrix interactions³⁷ including fibroblast activation and dysregulated collagen synthesis, are upregulated in adhesive capsulitis versus normal shoulder capsule⁴⁵ and the subacromial bursa²⁸ of patients with frozen shoulder. The clinical picture of significant shoulder pain has also been linked to increased cytokine production,²⁰ while several studies highlighted increased nerve ingrowth⁵² and neuronal mediators in frozen shoulder.^{16,49}

Alarmins (also known as danger signals) are endogenous molecules that are released into the extracellular milieu after infection or tissue injury and that signal cell and tissue damage.³ These molecules have been shown to be associated with cytokine activation and the influx of immune cells such as monocytes.³⁶ Alarmins share common functional characteristics; in addition to causing immune cell activation, alarmins are released rapidly during necrosis and apoptosis while retaining the potential for active secretion by immune cells and ultimately promoting tissue homeostasis⁴⁰; thus, modulating their activity may provide novel avenues to control a variety of inflammatory processes. It is now widely accepted that alarmins play a key role in the pathogenesis of many inflammatory diseases and not only initiate but amplify and sustain the inflammatory processes. The best characterized alarmins in musculoskeletal disease are high-mobility group protein B1 (HMGB1), S100 proteins (S100A8 and S100A9), heat shock proteins (HSPs), and interleukin 33 (IL-33). Elevated serum levels and expression of S100 proteins within inflamed synovium have been reported in rheumatoid arthritis,¹¹ psoriatic arthritis,²¹ and spondylarthropathy.²³ HMGB1 is expressed in synovial fluid and serum of patients with rheumatoid arthritis,¹⁵ while therapeutic blockade of HMGB1 prevents progression in mouse models of arthritis.³¹ Additionally, we have shown that IL-33 is an alarmin in early tendinopathy³⁴ with the ability to modulate inflammatory and matrix crosstalk and thus is likely to be important in the balance between reparation and degeneration in tissue repair.

To our knowledge, no previous studies have considered the potential contribution of alarmins in frozen shoulder. Thus, given the potential role of alarmins in soft tissue immunobiology, we designed a study to investigate whether alarmins might contribute to the pathogenic mechanisms of frozen shoulder. The aims of this study were to evaluate alarmin molecules in patients presenting with frozen shoulder and to assess any correlation of these molecules with patient symptoms.

METHODS

Study Design

A prospective case-control study was conducted at the Orthopaedic Research Institute, St George Hospital, Sydney, NSW, Australia, and the Institute of Infection, Immunity and Inflammation at the University of Glasgow, Scotland, UK. The study was conducted in accordance with ethics approval from Human Research Ethics Committee—Central Network, South East Health (HREC/96/55, HREC/14/130) and West of Scotland REC (REC14/WS/1035).

Inclusion and Exclusion Criteria

The study included patients with primary frozen shoulders who were receiving arthroscopic capsular releases. It also included a control group of patients with unstable shoulders undergoing arthroscopic stabilization; these patients were thought not to have primary shoulder disease affecting the shoulder capsule and they required glenohumeral arthroscopy, which was necessary to obtain tissue samples.

The diagnosis of frozen shoulder was made by use of Codman's modified criteria by Zuckerman and Rokito.⁵⁴ The inclusion criteria for the frozen shoulder population comprised a painful stiff shoulder with insidious onset, elevation restricted to 100° or less, passive external rotation 50% or less of the normal contralateral shoulder, loss of function of the affected arm, pain at night and inability to lie on the affected side, and unremarkable radiographic and ultrasonographic findings of the glenohumeral joint. The exclusion criteria included previous surgery of the involved shoulder and radiologic or arthroscopic signs of fracture, glenohumeral arthritis, history of shoulder trauma, and concomitant rotator cuff tear.

Sample Collection

Tissue specimens were excised from the shoulder capsule by the same surgeons (G.A.C.M., N.L.M.) during glenohumeral joint arthroscopy in patients undergoing either arthroscopic capsular release or stabilization surgery as previously described.¹⁹ The tissue was removed by meniscal basket forceps from the shoulder capsule of the rotator interval. The biopsied tissue was immediately fixed in 10% buffered formalin for 18 to 24 hours, dehydrated, and embedded in paraffin wax.

Pain and Stiffness Scores

On their first visit to the shoulder clinic, patients completed a modified L'Insalata questionnaire form,²⁵ which recorded subjective experience of pain frequency, pain severity, and stiffness. Patients answered questions regarding frequency of activity pain, night pain and extreme pain, severity of

resting pain, activity pain and night pain, and shoulder stiffness. Pain frequency was ranked by use of Likert scales as never (0), sometimes (1), monthly (2), weekly (3) or daily (4). Pain severity and stiffness were ranked as none (0), mild (1), moderate (2), severe (3), or very severe (4). The patient-ranked pain frequency and severity scores were summed to a maximum score of 12. The maximum patient-ranked stiffness was 4.

Immunohistochemistry

Samples for immunohistochemistry were immediately fixed in 10% formalin solution and stained with hematoxylin and eosin (H&E). Ten unstained sections were immunostained for the alarmin molecules IL-33 (Nessy-1, ALX-804-840-C100; Enzo Lifesciences), HMGB1 (Cambridge Bioscience), S100A8 (Abnova MAB7961), and S100A9 (Abnova PAB11470). Additionally, we used PGP9.5 (polyclonal rabbit anti-PGP9.5; Invitrogen) as a peripheral nerve marker, CD68 as a macrophage marker, and mast cell tryptase as a mast cell marker on the same 10 unstained samples. The unstained sections were dewaxed in an oven at 60°C for 35 minutes. They were then immersed in 2 changes of xylene for 5 minutes each and deparaffinated in 100%, 90%, and 70% ethanol solution for 2 cycles of 3 minutes each; thereafter, they were rehydrated in distilled water for 5 minutes. Sections were then immersed in 0.5% hydrogen peroxide made up in methanol for 30 minutes to block endogenous peroxidase activity and then tris-buffered Saline 0.05% with Tween (TBST) solution (pH 7.6). Endogenous peroxidase activity was quenched with 3% (vol/vol) H₂O₂, and nonspecific antibody binding was blocked with 2.5% horse serum in TBST buffer for 30 minutes. Antigen retrieval was performed in 0.01 M citrate buffer for 20 minutes in a microwave. After excess solution was removed, IL-33, HMGB1, S100A8, S100A9, and PGP9.5 antibodies were added at 5 µg/mL, 2 µg/mL, 3 µg/mL (S100A8 and A9), and 1 in 1000 dilution, respectively; dilutions were made up with 2.5% normal horse serum. The sections were left overnight to incubate at 4°C.

The sections were brought to room temperature for 30 minutes and washed in TBST solution for 5 minutes. The sections were then incubated in 3,3'-diaminobenzidine (DAB) solution (1 drop of DAB to 1 mL of DAB diluent) for 30 seconds and quickly washed with TBST solution. They were then dipped twice in hematoxylin, washed in water, dipped in Scott's solution to blue, and then dehydrated in 70% ethanol (30 seconds), 90% ethanol (1 minute), and 2 changes of 3 minutes in 100% ethanol. Then the sections were cleared in 2 changes of xylene for 3 minutes and mounted in DPX with a coverslip.

Microscopic Analysis

Tissue sections were examined under light microscopy at ×100 and ×400 magnifications and compared with the negative controls. Representative photomicrographs of the sections in each group were made. Two experienced examiners (J.C., N.L.M.) blinded to the identity of the stained sections performed the counting of each section.

Tissue analysis occurred in 2 stages. In the first stage, all samples were given a semiquantitative grade based on the percentage of positively stained cells (taken over the total number of cells in that field) in 10 random high-power fields. The following semiquantitative scoring (modified Bonar score) was used: grade 0, no staining; grade 1, mild, <10% of cells stained positive; grade 2, moderate, 10% to 20% of cells stained positive; grade 3, strong, >20% of cells stained positive. The mean of these values was analyzed by an unpaired Student *t* test.

In the second stage, 10 random high-power fields of the samples were analyzed at ×40 magnification, and cells in each field were counted manually. The mean percentage of positively stained cells was taken over the total number of cells per high-power field; similarly, the results were analyzed by an unpaired Student *t* test.

Statistical Analysis

Results are reported as the mean value and the standard error of mean. Comparisons between groups were made with 2-way paired Student *t* tests, Mann-Whitney *U* tests, and Kruskal-Wallis 1-way analysis of variance on ranks. On the basis of our previous immunohistochemical studies³⁵ and power calculations^{7,38} (power of 0.8 and beta error of 0.2), we identified that each group required 10 patient samples to detect a 20% difference in immunostaining for the various alarmin markers. Correlations were calculated with the Pearson coefficient.

RESULTS

Demographics

The samples of a total of 20 patients were included in the study. Of these 20 patients, 10 had adhesive capsulitis (5 females, 5 males; mean age 54 years; range, 42-65 years) and 10 had shoulder instability (2 females, 8 males; mean age 33 years; range, 18-46 years). The instability group was significantly younger than adhesive capsulitis group ($P < .01$). The mean number of glenohumeral joint injections was significantly ($P < .05$) greater in the frozen shoulder group compared with control (Table 1).

Pain and Stiffness Scores

Patient-ranked pain frequency ($P = .02$), pain severity ($P = .02$), and stiffness ($P = .04$) were significantly higher in the frozen shoulder group compared with the instability group (Figure 1). Shoulder motion was also significantly more restricted in external rotation, internal rotation, forward flexion, and abduction in patients with frozen shoulder compared with the instability group (Table 1).

Histologic Appearance of the Shoulder Capsule

The histologic appearance of the H&E-stained frozen shoulder capsule specimens showed densely packed collagen fibers

TABLE 1
Patient Demographics

	Adhesive Capsulitis (Frozen Shoulder)	Instability (Controls)
No. of patients	10	10
Age, mean (range), y	54 (42-65)	33 (18-46)
Sex, female:male, n	5:5	2:8
Duration of symptoms, mean \pm SEM, mo	6.3 \pm 3	3.9 \pm 2
Range of motion, mean \pm SEM, deg		
Forward flexion	80 \pm 12	160 \pm 8
Abduction	62 \pm 10	145 \pm 10
External rotation	11 \pm 5	60 \pm 15
No. of steroid injections before surgery, mean \pm SEM	1.6 \pm 0.2	0.4 \pm 0.1

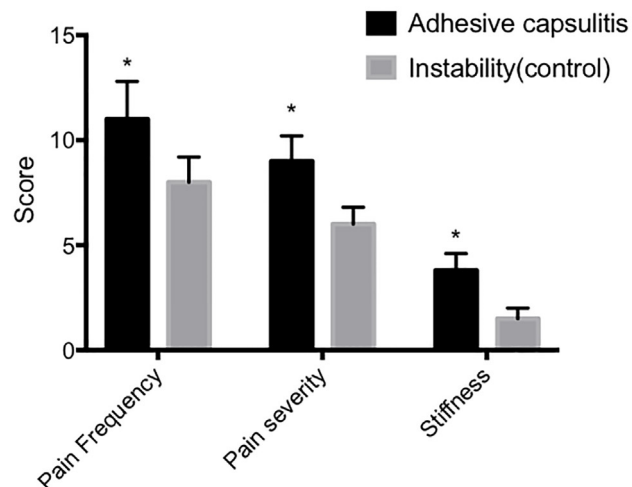
and fibroblastic proliferation within the fibrous stroma compared with the controls (Figure 2A). Large numbers of capillaries and venules were seen in the subsynovium of frozen shoulder samples compared with the controls. Capsule specimens from 4 instability patients also exhibited few capillaries and venules in the subsynovium, whereas 2 had increased vascularity.

Increased Alarmin Expression and Neoinnervation in Adhesive Capsulitis

Significantly greater expression of the alarmin molecules HMGB1, IL-33, S100A8, and S100A9 was seen in the frozen shoulder group compared with the control group ($P < .05$) (Figure 2B). HMGB1 and IL-33 were mainly localized to stromal cells, while the S100 molecules were less abundant and wholly localized to immune cells (Figure 2B). Further subanalysis of tissue distribution revealed that HMGB1 was most abundantly expressed in adhesive capsulitis with approximately 50% of cells positive versus 26% in control tissues (Figure 3), followed by IL-33 (25% vs 14% control, $P < .05$). In keeping with the localization of the S100 proteins to immune cells, only 4% of cells were positive for S100A8 versus 1% in control tissues ($P < .05$) and 5% were positive for S100A9 versus 1% in control tissues ($P < .05$) (Figure 3). Further subanalysis using back-to-back staining with the macrophage marker CD68 revealed that both S100A8 and S100A9 were localized to macrophage cells.

Neoinnervation and HMGB1 Expression Correlate to Patient Pain

Patients with frozen shoulder had significantly greater neoinnervation ($P < .01$) compared with the control group (Figures 2 and 3). Increased neoinnervation positively correlated with the frequency of severity of pain ($r = 0.99$, $P < .05$) and extreme pain ($r = 0.93$, $P < .05$) in patients with frozen shoulder versus control patients (Figure 4B).



Patient Demographics		
	Adhesive Capsulitis	Instability (controls)
No. of Patients	10	10
Mean Age (years), (range)	54 (42-65)	33 (18-46)
Gender (F:M)	5:5	2:8
Duration of symptoms (months)	6.3 \pm 3	3.9 \pm 2
Range of motion (degrees)		
Forward flexion	80 \pm 12	160 \pm 8
Abduction	62 \pm 10	145 \pm 10
External Rotation	11 \pm 5	60 \pm 15
Mean number of steroid injections prior to surgery	1.6 \pm 0.2	0.4 \pm 0.1

Figure 1. (A) Patient-ranked shoulder pain frequency, pain severity, and stiffness scores in patients with frozen shoulder ($n = 10$) compared with those with instability ($n = 10$). Data show mean with the standard error of the mean (error bars). * $P < .05$, Kruskal-Wallis-Dunn multiple comparisons test. (B) Patient demographics and shoulder range of motion in patients with frozen shoulder ($n = 10$) compared with those with instability ($n = 10$). [AQ: 1] Yes please delete 1B

Increased HMGB1 expression positively correlated with the frequency of night pain ($r = 0.97$, $P < .05$) and extreme pain ($r = 0.96$, $P < .05$) in patients with frozen shoulder versus control patients (Figure 4A). We found no correlation between alarmin tissue staining and the number of steroid injections ($r = 0.2$, $P = .8$) preoperatively or coexisting metabolic issues (diabetes or thyroid disease; $r = 0.01$, $P = .7$).

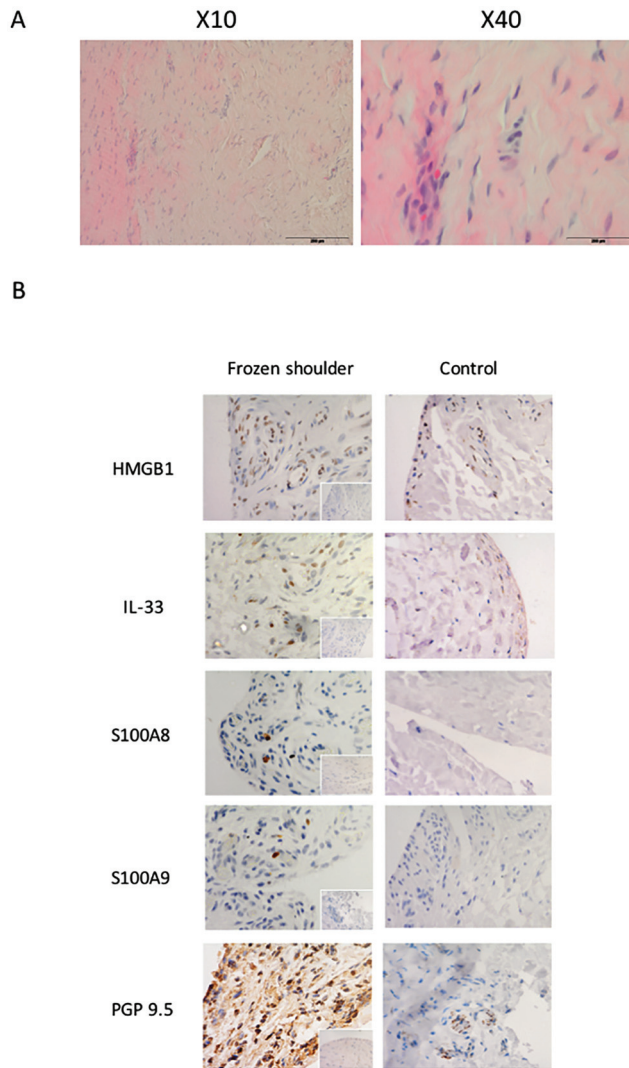


Figure 2. (A) Hematoxylin and eosin (H&E) sections from frozen shoulder showing $\times 10$ and $\times 40$ representative images of frozen shoulder tissue. (B) Immunostaining for the alarmins high-mobility group protein B1 (HMGB1), interleukin 33 (IL-33), S100A8, and S100A9 and peripheral nerve (PGP9.5) markers in frozen shoulder compared with control. All slides represent $\times 200$ magnification, and isotype controls (immunoglobulin G) are shown in the bottom right corner of the frozen shoulder images.

DISCUSSION

This study, which is the first to investigate the expression of alarmin molecules in frozen shoulder capsule, has demonstrated the presence of HMGB1, IL-33, S100A8, and S100A9 in shoulder capsule tissue of patients with adhesive capsulitis. Furthermore, we demonstrate that patient-reported pain in frozen shoulder directly correlates with increased neoinnervation and HMGB1 expression.

HMGB1 is a nuclear protein released in nonprogrammed cell death and secreted by immune⁴⁶ and stromal cells,

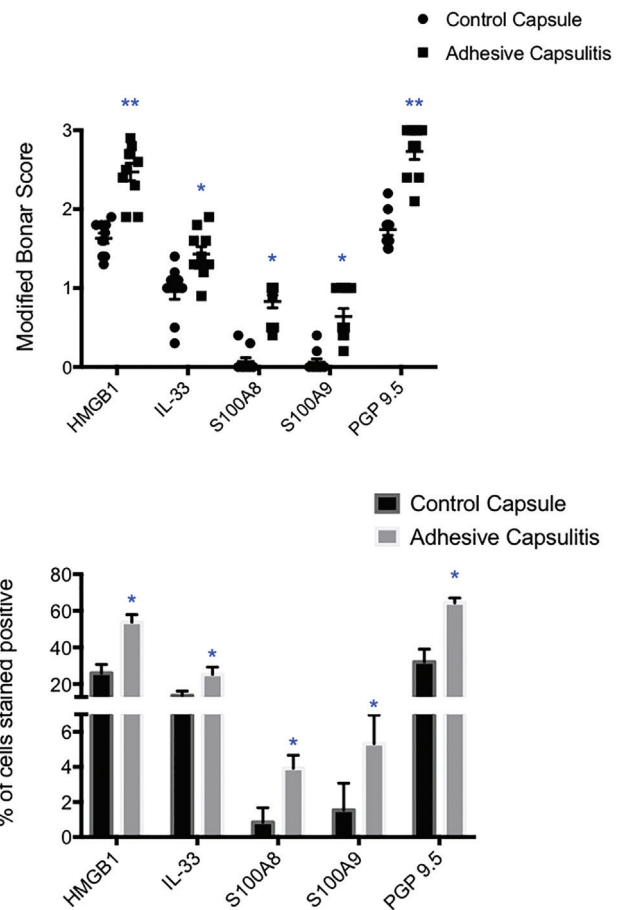


Figure 3. (A) Graph illustrating modified Bonar score for samples of human frozen shoulder for expression of high-mobility group protein B1 (HMGB1), interleukin 33 (IL-33), S100A8, S100A9, and PGP9.5 markers, with mean and SEM shown. $n = 10$ for frozen shoulder, $n = 10$ for instability control. Modified Bonar scoring system depicts mean score per sample based on 10 high-power fields. 0 = no staining; 1 = $<10\%$; 2 = $10\%-20\%$; and 3 = $>20\%$ positive staining of cells per high-power field. $*P < .05$, $**P < .01$ (Student t test). (B) Quantitative expression of HMGB1, IL-33, S100A8, S100A9, and PGP9.5 depicting mean cells per sample based on 10 high-power fields. $n = 10$ for frozen shoulder, $n = 10$ for instability control capsule (Student t test).

including fibroblasts.^{29,42} As well, HMGB1 can be induced by damage-associated molecular patterns (DAMPs), cytokines, and certain states of cellular stress.³⁶ Conditions associated with an increase in HMGB1 include rheumatoid arthritis, systemic lupus erythematosus, and Sjögren syndrome,³⁶ whereas exposure of synovial fluid macrophages to HMGB1 results in the release of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6.^{26,27,48} In particular, HMGB1 acts as a DAMP after its release^{6,32} upon stress conditions, which, in turn, are considered crucial to the onset and perpetuation of frozen shoulder.^{44,17} Thus, our results demonstrating significantly increased HMGB1 expression localized

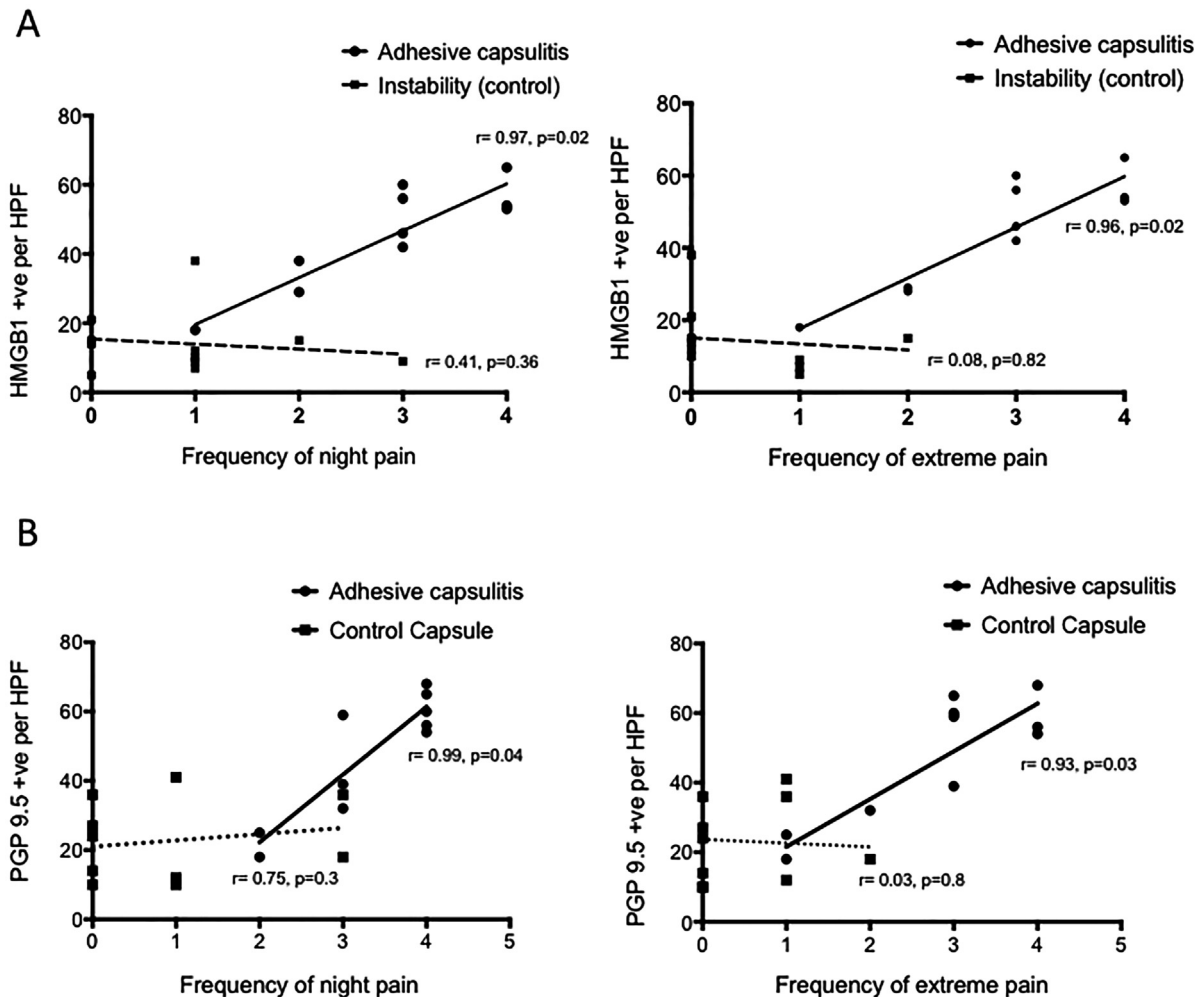


Figure 4. (A) High-mobility group protein B1 (HMGB1) and (B) PGP9.5 count per high-power field (HPF) correlated with patient-reported frequency of night pain and frequency of extreme pain in patients with frozen shoulder versus control as shown by the indicated Pearson coefficient.

to stromal cells in frozen shoulder suggest that the resident stromal cells (fibroblasts and endothelial cells) may act as damage-sensing cells that release HMGB1 upon stress, perpetuating the inflammatory tissue response seen in patients. Moreover, agents targeting HMGB1 have delivered promising in vitro and in vivo data,^{10,39,47} particularly altering matrix regulation, suggesting that HMGB1 may offer therapeutic utility by providing a novel pathway to target in frozen shoulder.

Recently, HMGB1 was shown to be secreted not only by leukocytes and stromal cells but also by irritated or damaged neurons, which release HMGB1 into the extracellular space.⁵⁰ Experimental studies suggest that HMGB1 is released both peripherally and spinally in models of pathologic pain and can drive nociceptive signaling via actions on sensory neuronal and immune cells.¹ Of importance, systemic and intrathecal administration of HMGB1-blocking agents reverses pain-like behavior, indicating that HMGB1 has pronociceptive properties in both the peripheral and central nervous systems.⁵¹ Our data

suggest that increased expression of HMGB1 is directly associated with increased patient-reported pain and as such may be a molecular mediator of the significant pain associated with frozen shoulder. More studies are now required to elucidate the underlying mechanisms involving HMGB1 upregulation to understand whether this danger signal is involved in shoulder inflammation and pain in patients with frozen shoulder. Additionally, our results suggest that HMGB1 may be a promising biomarker for frozen shoulder diagnosis and patient outcomes. We have previously used PGP9.5 as a peripheral nerve marker to quantify the degree of neoinnervation in soft tissue disorders such as frozen shoulder¹⁹ and calcific tendinopathy¹⁴ and link its expression to patient-reported pain. Our results in this cohort of patients further suggest that the significant pain associated with frozen shoulder is linked to increased peripheral nerve ingrowth. Future work should therefore explore the molecular mechanisms involved in neoinnervation and inflammation in frozen shoulder.

IL-33 induces hematopoietic cells such as macrophages, mast cells, and neutrophils to produce cytokines such as IL-6, TNF- α , and IL-1 β . IL-33 has also been shown to increase vascular permeability and to induce chemotaxis, angiogenesis, and endothelial cell activation.^{2,9} Moreover elevated serum IL-33 has been found in autoimmune musculoskeletal diseases⁴¹ and fibrotic conditions such as systemic sclerosis and liver fibrosis,^{33,43,53} suggesting that while IL-33 promotes tissue repair, there is evidence of maladaptation resulting in pathologic changes and fibrosis.³⁰ We have previously shown that IL-33 is mainly expressed in the stromal tissue compartment in tendinopathy and is crucial in driving inflammatory and matrix changes toward degenerative disease.³⁴ Our finding of elevated nuclear expression of IL-33 in frozen shoulder stromal cells is therefore likely to signify that IL-33 is involved in the key pathologic features of angiogenesis and capsular tissue fibrosis. Furthermore, given the role of IL-33 in immune cell recruitment and activation,²⁴ particularly macrophages and mast cells, which are found in abundance in human frozen shoulder biopsy specimens,¹⁶ IL-33 may be an important immune cell coordinator driving the inflammatory component of the frozen shoulder.

In contrast, S100A8 and S100A9 are expressed to a much lesser extent in frozen shoulder and are localized around immune cells under histologic analysis. S100A8 and S100A9 were initially identified in the context of rheumatoid arthritis,¹² with activated phagocytes expressing these S100 proteins in the inflammatory lesions in synovium.¹³ In patients with active disease, S100A8 and S100A9 were expressed in macrophage-like cells within the lining layer, showing altered activation and differentiation.⁸ In view of this, we carried out further immunostaining with a macrophage marker in a subgroup of patients with frozen shoulder, which revealed that the S100 protein expression was localized to macrophages. Thus, in frozen shoulder it appears that the S100 proteins are upregulated in macrophages and are therefore likely to be involved in macrophage differentiation and recruitment, further supporting the concept that frozen shoulder has a strong inflammatory and immunological origin.

Our study has inherent limitations. The sample size was small; however, our previous studies^{14,19,38} and power analysis confirmed that 10 patient samples per group were sufficient, which gives us confidence that the changes seen represent a biologically relevant change in protein expression. Additionally, a selection bias was present in that we studied only patients with frozen shoulder that warranted a surgical procedure, and thus we did not include asymptomatic patients or patients who had less intense pain with frozen shoulder. Furthermore, the patients in the control group were younger, were predominately male, and had shorter duration of symptoms compared with the frozen shoulder group, which is an unfortunate consequence of the availability of human control tissues. Two surgeons were involved in the collection of samples, giving rise to the possibility that surgical technique and biopsies had some user-related differences. Despite this, the same method of obtaining samples was used to reduce the surgeon-related bias. This study ensured that comparison occurred only

between shoulder capsule samples of patients with adhesive capsulitis and shoulder capsules samples of patients with instability. This allowed a comparison between the same type of tissue and removed the possibility that pathologic abnormalities in other tissue types, such as tendons, could confound the study. The exclusion of patients with concurrent pathologic conditions such as osteoarthritis and rheumatoid arthritis also allowed better isolation of cases to adhesive capsulitis in the shoulder. As such, results of immunoreactivity were less likely to be affected by other comorbidities.

CONCLUSION

This study has shown for the first time that the alarmins HMGB1, IL-33, S100A8, and S100A9 are elevated in the shoulder capsule of patients with frozen shoulder compared with controls while furthermore confirming significantly increased neoinnervation linked to patient-reported pain. These data are consistent with the hypothesis that alarmins play a role in frozen shoulder and could explain the cellular mechanisms behind capsular fibrosis and nerve growth.

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Yes all correct

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